RS=-SR + SO²
$$\rightarrow$$
 RS=-SO₃ + RS

Obtaining agent (tetrathionate)

RS + SR + SO² \rightarrow RS + RS + SO² \rightarrow RS + O₃S + S + SO² \rightarrow RS +

RS-S-SO
$$_3$$
 + SO $_3$ + S-SO $_3$ + S₂O $_3$

Proposed reaction for oxidative sulfitolysis

Figure 1A.

Cleavage of disulfide bond by sodium sulfite to form the S-sulfo derivative

Figure 1B.

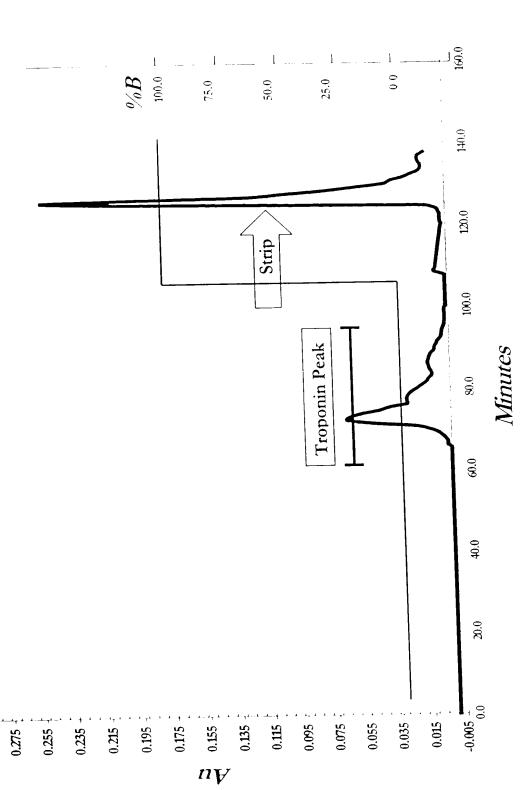
Preparation and Washing of TnI-containing Inclusion Bodies

Disperse Frozen Cell Pellet (150 g) in 1.5 Liter (10x w/v) 4°C 50 mM Sodium Acetate, 2 mM EDTA, pH 6.0 Lyse Cells w/Microfluidizer, 10,000 psig, 2 Passes, 10°C Centrifuge Lysed Cellular Material 12,000 G, 30 Minutes, 4°C Decant Supernatant, Disperse Pellet in 1.5 Liter 50 mM Sodium Acetate, 2 mM EDTA, 1% Triton X-100, pH 6 Centrifuge 12,000 G, 30 Minutes, 4°C Decant Supernatant, Disperse Pellet in 1.5 Liter 50 mM Sodium Acetate, 2 mM EDTA, 0.5M NaCl, pH 6.0 Centrifuge 12,000 G, 30 Minutes, 4°C Decant Supernatant, Disperse Pellet in 1.5 Liter 50 mM Sodium Acetate, 2 mM EDTA, pH 6.0 Centrifuge 12,000 G, 30 Minutes, 4°C Decant Supernatant, Disperse Pellet in 0.2 Liter 50 mM Sodium Acetate, 2 mM EDTA, pH 6.0 Centrifuge 12,000 G, 30 Minutes, 4°C

Freeze Pellet for Further Processing, -70°C

Summary of rTroponin-I Preparation – 3L5 (050900-051200) Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5, 100mM NaCl. Buffer B: 6M urea, 25mM Tris HCl, pH 3L Q-Sepharose FF column. 7.5, 2M NaCl. Gradient: Step; 0° o B for the flow through then to 100% B for strip and cleaning. Flow rate: 150ml/min. 3000 ml applied = **10-fold** concentration (UF) to 300 ml. Buffer exchange (DF) against 5L. 6M urea, 25mM Tris-HCl, pH 7.5. UF/DF; 0.2 ft² Pall Omega cassette. 290 ml as the final retentate volume...load for 300 ml Q-Sepharose FF column. Buffer A: 6M urea, 25mM Tris-HCl, pH Buffer B: 6M urea, 25mM Tris-HCl, pH 7.5, 2M NaCl. 300 ml Q-Sepharose FF column. Gradient: Step; 4% B for elution and 50% for cleaning. Flow rate: 20ml/min. Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5, 1M (NH₄)₂SO₄. Buffer B: 6M urea, 25mM Tris-HCl, pH 60 ml Toyopearl 650M HIC (phenyl) column. Gradient: Step; 100% B for flow-through to 0% B for strip and cleaning. Flow rate: 10ml/min. 550 ml applied Buffer exchange (DF) against 5L 25mM citrate, pH 3.0, 150mM NaCl. 200 ml as the final retentate volume UF/DF; 0.2 ft² Pall Omega cassette. 100, 1 ml aliquots and 25, 4 ml aliquots. Final 3L5 product.

Figure 3



0.295

Figure 4

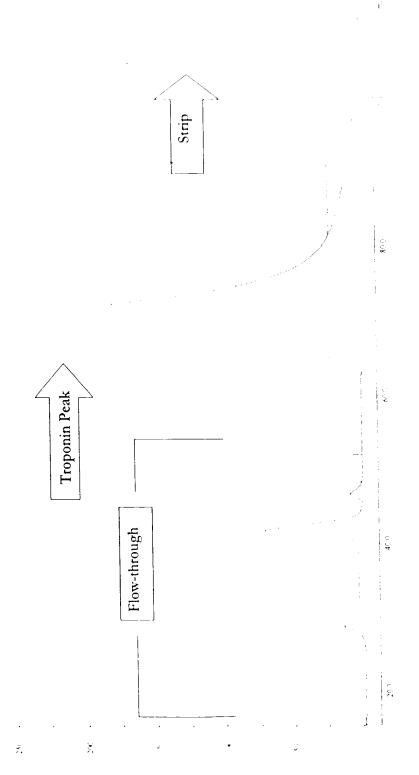


Figure 5

SDS-PAGE Analysis Troponin Lot 3L5

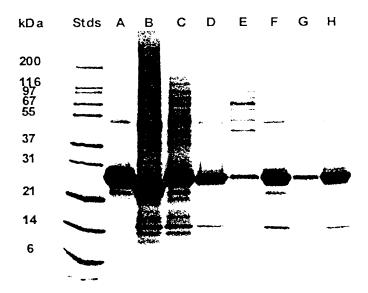


Figure 6

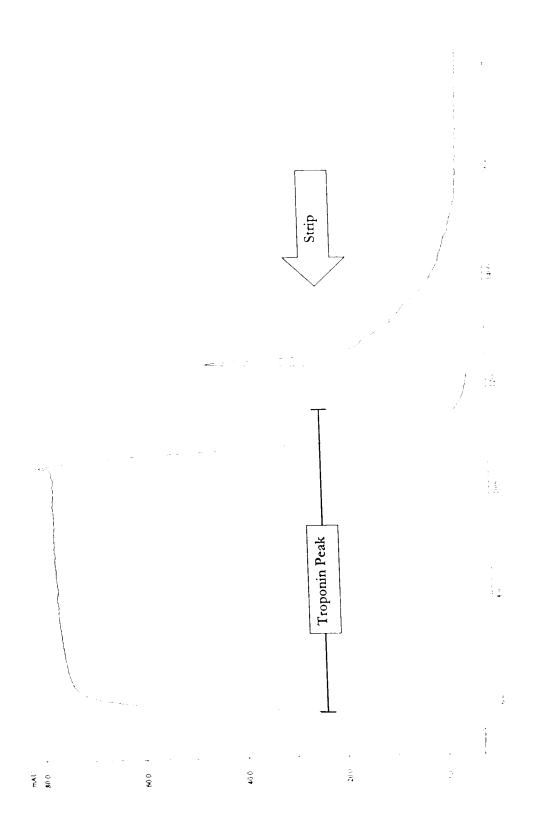


Figure 7

SDS-PAGE Analysis Troponin Lot 3L5 Hydrophobic Interaction Chromatography 16% Tris-glycine Gel, Non-reducing, 5/15/00

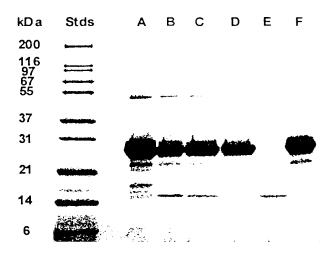


Figure 8

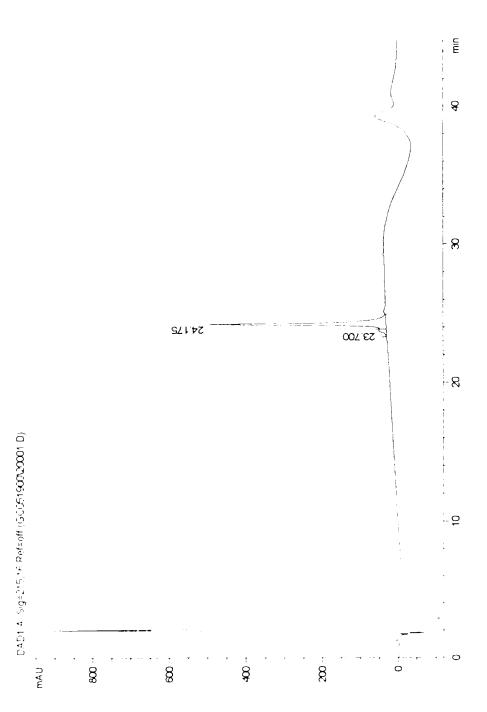


Figure (

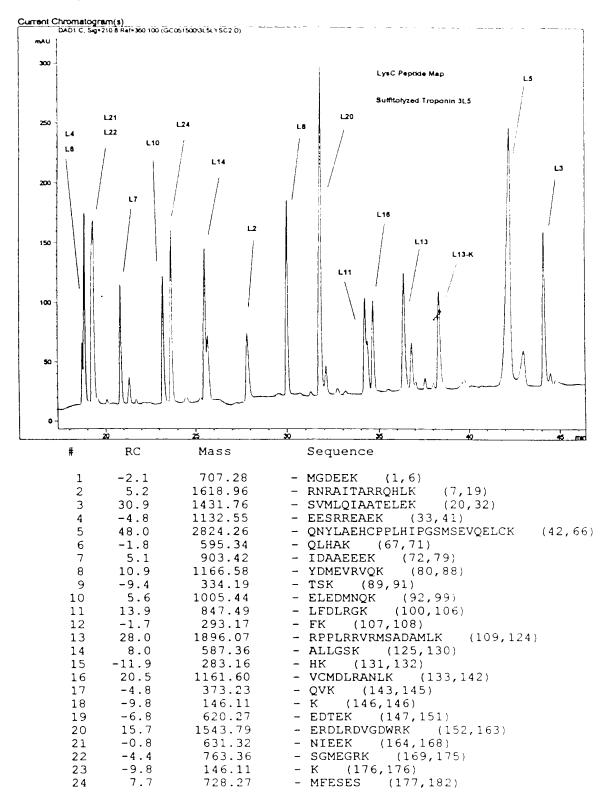


Figure 10

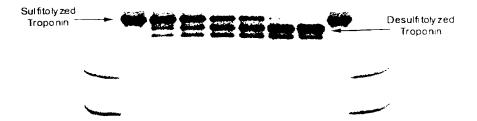


Figure 11